OBSERVATIONS ON AZOTOBACTER

THE group of bacteria having the capacity of using free atmospheric nitrogen in their metabolic processes consists of three general types: (1) Those associated with the nodule formation of legumes (*Ps. radicicola*); (2) large bacilli which produce spores located in the center of the cell, causing an increased diameter of the cell at that point (*Clostridium pasteraneum*); and (3) a form displaying considerable variation in size and shape, which, according to original descriptions, is without endospores (*Azotobacter* sp.).

The discovery of the extraordinary ability of these organisms to secure a supply of nitrogen from the air brought them into immediate prominence as objects of systematic study. The nitrogen-assimilating property was first detected in the *radicicola*, and hence the earlier studies were concentrated upon this organism. After the isolation of the other two forms, however, they assumed quite as much importance as had attached to the legume organism. In fact, recently more attention has been given to the former than to the latter. This has been especially true of the *Azotobacter*. The *Clostridium* has never been so inviting as either of the others; perhaps because of its morphological uniformity. Then, too, it appears to have less nitrogen-gathering power.

At various times, while working with other phases of the problem of soil bacteriology, I have attempted to isolate the *Azotobacter* from our local soils and study it rather intensively. On several occasions these efforts have resulted in the securing of pure cultures of the bacillus. So far as the observations went, however, no peculiarities were presented which had not previously been noted by other investigators. During the year 1913 a culture was secured which showed some striking qualities. These attracted immediate attention and led to an extensive study.

Soon after the isolation of *Azotobacter croococcum* by Beyerinck, other investigators gave descriptions of five or six types of *Azotobacter* which they regarded as distinct species. One of these forms was given the name *Azotobacter vinelandii* by Dr. J. G. Lipman, who isolated and described it. The chief basis upon which this species was established is the quality of pigment production. In this case the color of the pigment is a distinct yellow, as contrasted with the heavy brown pigment of *A. croococcum*.

From the outset the bacillus under consideration here has been regarded as a variety of *A. vinelandii*. In many respects, however, our type differs so markedly from the original description of *A. vinelandii* as to create considerable doubt as to the identity of the species. Nevertheless, I am still disposed to regard it as a mere variety within this group. As a matter of fact, the recent work of Prazmowski and others tends to eliminate the various species of this organism and regard them all as one species consisting of several varieties. My own observations lead me to favor this contention. The pigment color of the culture in my laboratory shows considerable variation, ranging from yellow to brown. This seems to depend largely upon the medium used.

The following comparison will show the chief points in which our type varies from the species description:

Features Considered	Original Description	Observation Made in the Present Investigation
Mannite agar plates	Colonies 4 mm. in 4 days.	Colonies 6–8 mm. in 4 days.
Mannite agar plates	Colonies whitish.	Colonies yellow.
Glucose solution	Surface film.	No surface film.
Mannite solution	Yellow pigment.	No pigment.
Glucose agar stab	White.	Yellow.
Mannite agar stab	White.	Yellow.
Potato	Dirty white.	Pink, young; yellow, old.
Spore formation	Absent.	Present.
Thermal death point	80°–85° C., 5 min.	90°–95° C., 5 min.

Probably nothing else in this comparison is so significant as the disagreement in regard to the spore production and the corresponding high thermal death point of our cultures.

Beyerinck, in his original observations on *Azotobacter croococcum,* failed to detect the presence of spores. Nevertheless, in 1911 E. Mencl[1] demonstrated their presence in this species. It was recognized early in the study of *Azotobacter* that they are very resistant to drying and other adverse conditions, which fact aroused the suspicion that spores are produced. The morphological irregularity and change of form under different cultural conditions obscured the true nature of the case until the date mentioned above. If all *Azotobacter* are to be regarded as one species, my observation of spores is, of course, only a repetition of observations made recently by several investigators.

In the cultures with which I worked the organisms attained very great size, and showed in many cases a striking resemblance to budding, similar to that observed in yeast cells. At such times the cell is well filled with refractive bodies which do not stain readily with the analine dyes. Such bodies have generally been looked upon as fat, but the fact that by special effort they can be stained with methylene blue led to some doubt as to their fatty nature. Efforts were made to stain these bodies with the ordinary fat stains, such as Soudan 3 and Sharlac red, but the results were all negative. *Azotobacter* were then grown on potato in sufficient quantities to secure a sample large enough for ether extraction. Potatoes were cut in thin slices and sterilized in petri dishes, and then inoculated. The growth was excellent, and a considerable mass of this was secured by scraping from the surface.

The material thus secured was placed in a separatory funnel and treated with pure ether for twenty-four hours without heat. The funnel was shaken several times during the extraction and then allowed to stand for sedimentation. Several cubic centimeters of the clear solution were then drawn into a weighed platinum dish and evaporated to dryness. It was found that a sediment detectible by weight was left in the dish. This may have been a mixture of fats, gums and resins, or possibly any one of these.

The funnel in which the extraction was made was set aside with the residue of the bacterial mass and a thin layer of ether which overlay the mass. No attention was given to it for about two weeks. When it was examined there was a brownish layer on the surface of the ether. This layer gave one the impression that it consisted of a bacterial growth. Ether has, of course, been regarded as a disinfectant, and although it has never been thought of as having great germicidal power, yet in a high concentration one would certainly be surprised if it should not be found sufficient to inhibit all growth.

Cover-glass preparations were made from the scum on the ether in the funnel referred to, and it was found to contain large numbers of bacteria. These might have come from the original mass from the potato and been dead at the time they were taken from the ether. At the same time that the microscopic preparations were made, however, inoculations on agar were placed in the thermostat for incubation. At the end of twenty-four hours, when the agar tubes were examined, they were found to have a good growth on them, stains from which showed that it consisted of *Azotobacter.* It was evident, therefore, that practically pure ether had not killed this organism. It was also made reasonably certain by this observation that the scum on the ether in the funnel consisted of living *Azotobacter.*

It remained to be shown that the organisms were actually multiplying in the solution. This was accomplished in the following way: Small Ehrlenmeyer flasks were supplied with sufficient Squibb's pure ether to make a thin layer on the bottom of the flask and a small particle of the potato culture was introduced into them. These were set aside at room temperature for development, and at the end of a week or ten days a decided growth could be detected.

Another method that was employed to test

[1] *Arch. Protistenk.,* 22 (1911), No. 1, pp. 1–18.

the ability of *Azotobacter* to grow in the presence of ether consisted in placing cylinders of potato in test tubes having plugs of absorbent cotton in the bottom saturated with pure ether. The slant of the potato was inoculated with the organism and incubated at room temperature. These cultures almost invariably had a growth at the end of from eight to ten days. The growth on potato had a decided stringiness. Stains from such a mass revealed a dense zooglea.

In the case of the cultures in liquid ether the only apparent source of carbon is the ether itself, and the bacteria are therefore under the necessity of using this in their metabolic processes. When the flasks containing old cultures were examined from day to day it was possible to detect what appeared to be the odor of alcohol and ether alternately. The successive hydration of ether and dehydration of alcohol would account for this phenomenon, but the probability of bacteria being able to induce these changes is certainly extremely remote. The oxidation of ether has recently been shown to result in the formation of aldehyde and acetic acid. Any attempt, however, to explain the nature of the process taking place in the flasks would be mere speculation, since the matter has not been experimentally investigated.

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KNOXVILLE, TENN.

THE ROYAL SOCIETY OF CANADA

THE thirty-fourth annual meeting of the Royal Society of Canada was held in Ottawa, May 24 to May 27, inclusive, under the presidency of Sir Adolphe B. Routhier. The attendance was one of the largest in the history of the society, founded in 1882 by the Marquis of Lorne, at the time governor general of Canada. The four sections into which this national society is divided met under their respective presidents: Section I., French literature, history, archeology, etc. (in French); Section II., English literature, history, archeology, etc. In this section the following papers are of scientific value: "Some Notes upon the Discovery of a Prehistoric Human Skeleton in British Columbia," by Charles Hill-Tout. This skeleton came "from Undisturbed Strata in the white silts of the Interior Plateau" of that province, near Kamloops, and places the prehistoric history of man in western Canada back to the glacial period. "Social Organization of the West Coast Tribes," by Professor Adam Shortt, C.M.G., also forms an interesting study.

Section III., dealing with the mathematical, physical and chemical sciences, comprises numerous papers of special interest and value.

Professor R. F. Ruttan (McGill University), discussed "The Chemistry of Adipocere."

This paper deals with the changes in animal fats, as the result of prolonged action of moisture with the exclusion of air. The adipocere studied was found in a recent Post-Tertiary deposit of wet soil near Ormstown, Quebec. The material had the general character and appearance of soft chalk to the touch.

Another paper by Dr. Ruttan was entitled "Glycol Esters of the Fat Acids," pointing out a new series of fats formed by the replacement of ethylene glycol for the glycerol of ordinary fats.

Dr. Harding presented the result of investigations by him and Messrs. A. R. Maclean and F. H. S. Warneford, on "The Ninhydrin Reaction," being a critical study of this reaction for alpha amine acids, its quantitative relations and the chemistry of the color produced.

Then followed numerous contributions in the physical and mathematical sciences, and in astronomy, spectroscopy, electricity, metallurgy, meteorology, etc. These include:

A Comparison of Radium Standard Solutions: J. MORRAN. Presented by PROFESSOR A. S. EVE, F.R.S.C.

Notes on the Penetrating Radiation from the Earth: PROFESSOR A. S. EVE, F.R.S.C.

Some Experiments on the Thermionic Current: PROFESSOR A. S. EVE, F.R.S.C.

The Solar Rotation: DR. J. S. PLASKETT, F.R.S.C.

This paper gives the values of the spectroscopic determination of the Solar Rotation from plates made at Ottawa in the years 1911, 1912, 1913. A summary of the rotation values at different latitudes, the formula connecting the variation of velocity with latitude and discussions of other important aspects of the question was given.

The Determination of the Distance of the Nearer Stars from their Proper Motions and Radial Velocities: REYNOLD K. YOUNG, Ph.D. Presented by DR. J. S. PLASKETT, F.R.S.C.

From 167 stars whose parallax, radial velocity and proper motion are known, the direction and magnitude of the solar motion was found. The mean distance of the stars was evaluated by a comparison of the mean radial velocity and mean